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Abstracts

The use of polymerase chain reaction (PCR) in the detection and characterization of HBV variants

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In hepatitis B diagnostics the PCR is an established method for the assessment of patients' infectivity, for the therapeutic evaluation of interferon treatment and to assist in differentiating serological constellations.
Studies from various groups describe the occurrence of variants chronic hepatitis B in all four HBV genes (predominantly in the pre-C/C- and pre-S/S regions). Virus variants occur during fulminant and chronic, as well as during clinically asymptomatic courses. The significance of HBV variants, their selection mechanisms and influence on chronic liver disease are not well understood[1,2]. Kidney transplant (NTX) recipients exhibit a progressive course of chronic HBV infection with development of liver cirrhosis (LC) and death due to liver failure more often than hemodialysis patients[3,4].
We investigated whether in NTX recipients with a long-term transplant and pre-existing chronic hepatitis HBV, core deletion mutants with possible pathogenic significance emerge under immunosuppression. HBV DNA from serum of kidney transplant recipients was amplified by nested PCR using primers from the C gene[5]. The PCR products were subsequently separated in a 2% agarose gel. For cloning purposes the complete C gene, which codes for nucleocapsid and HBeAg, of selected probes was amplified by the use of two C-region-flanking primers (sense primer: 1767-1788; TTTGTACTAGGAGGCTGTAGGC and antisense primer 2510-2487; AGGTACAGTAGAAGAATAAAGCCC). An aliquot of the PCR products was ligated and cloned into the vector pCR 2 (Invitrogen) according to the manufacturer's instructions. Sequencing was performed with the Sequenase kit (USB).
During virological monitoring of chronically HBV-infected kidney transplant recipients using a PCR that amplifies most of the core gene, HBV variants were found in 9 of 24 cases. PCR revealed one or more shorter fragments with a higher mobility than the normal core band which was also present. After cloning and sequencing these shorter fragments were identified as HBV variants with deletions (24–130 nucleotides) in the central core region between nt 2120–2234. The open reading frame usually stayed intact. Clinically, the occurrence of core deletion mutants was almost always associated with severe progressive liver damage (cirrhosis, portal decompensation). The follow-up showed that core deletion mutants usually occurred 1–2 years before hepatic decompensation. An expansion of these deletion mutants was seen in seven of the patients during an observation period of about 2 years including one or more episodes of ascitic decompensation. Five of the patients died from liver cirrhosis. In the other 15 patients, three of which had liver cirrhosis, no C deletion mutants were detected at any time. None of these patients died.
HBV core deletion variants can be screened by a fast and simple PCR. Under immunosuppression an HBV subpopulation with extensive deletions in the central part of the core gene is selected and a coincident rapid progression of liver damage is observed. Our results suggest a casual relationship between the occurrence of core deletion mutants and terminal liver failure. An elevation of virus replication coupled with a direct cytopathic effect of aberrant C gene products could play an important role. Furthermore, the results indicate that selective mechanisms unrelated to the immune response may favour the production of these variants.

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